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Migration of Donor MHC Class II$^+$ Cells and Increase in Apoptosis: Correlate to Graft Outcome after Heart and Liver Transplantation

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We previously showed that short-course tacrolimus immunosuppression prolongs allograft survival for >100 days in rats. Histopathological examination of long-surviving allografts revealed severe chronic rejections with obliterative arteriopathy, lymphohitiocytic interstitial inflammation, patchy interstitial fibrosis, and atrophy of parenchymal elements in heart grafts; however, liver grafts were intact.[1,2] Passenger leukocytes, normal constituents of all whole organs, migrate after transplantation and produce microchimerism, which is suggested to be essential for the sustained survival of allografts.[3] One of the possible roles of donor passenger leukocytes is to regulate alloreactive recipient T cells via activation-induced apoptosis. This study analyzed the frequency and nature of migrating donor leukocytes and apoptosis after liver and heart transplantation.

METHODS

Brown Norway (BN) recipients underwent heterotopic heart (HTx) or orthotopic liver transplantation (OLTx) from Lewis (LEW) donors and were sacrificed at various predetermined time points (n = 2 to 6 for each time point).[4] Recipients were treated with a short course of tacrolimus (1.0 mg/kg/d) on days 0 to 13, 20, and 27. Apoptotic cells (TUNEL assay) and mRNA levels for cytokines and apoptotic signals (RNase protection assay) were examined in recipient spleens and allografts.[2] Donor cells were determined by flow cytometry (LEW MHC class I, MAb 163) and immunohistochemistry (LEW MHC class II, MAb L21-6).[5]

RESULTS

Donor MHC class I$^+$ cells accounted for 4% to 7% of splenocytes early after OLTx but were not detected after HTx by flow cytometry (Table 1). Donor MHC class II$^+$ cells were eminently identified at the periphery of the splenic periarterial lymphatic sheath and marginal zone with a frequency 10-fold more after OLTx than after HTx. Accordingly, splenic BrdU$^+$ cells dramatically increased after OLTx, followed by an increase in TUNEL$^+$ cells and mRNA for apoptotic signals in 14 days. Interestingly, however, the upregulation of splenic mRNA for IL-2 and gamma-IFN was more prominent after HTx than after OLTx. In heart allografts there was an upregulation of mRNA for IL-2 and gamma-IFN, with an increase in BrdU$^+$ infiltrates. In contrast, liver grafts showed an increase in TUNEL$^+$ cells and an upregulation of mRNA for apoptosis.

Table 1. Development of Chronic Allograft Rejection and Early Changes in Cytokine Responses and Frequency of Proliferation and Apoptosis in Allografts and Recipient Spleens*

Group	POD	HTx	OLTx
Chronic allograft rejection	100	+++	–
Spleen			
Donor MHC II$^+$ (cells/HPF)	3/7	0.16/0.02	10.6/1.7
Donor MHC I$^+$ (%)	7	0.0	5.4
IL-2 (%/GAPDH)	3	2.69	0.00
IFN-γ	3	6.46	2.6
IL-4	3	2.82	2.93
IL-10	3	5.00	3.01
Fas	14	3.44	7.41
FasL	14	2.94	7.36
Caspase 3	14	10.03	21.90
BrdU$^+$ (cells/HPF)	3/7	30.1/39.9	94.8/65.5
TUNEL	14	12.1	25.5
Graft			
IL-2 (%/GAPDH)	3	1.44	0.17
IFN-γ	3	1.54	0.29
IL-4	3	0.58	0.83
IL-10	3	0.62	2.27
Fas	14	3.76	5.87
FasL	14	1.53	1.36
Caspase 3	14	8.81	19.21
BrdU$^+$ (cells/HPF)	7	21.4	6.6
TUNEL	14	2.6	7.5

All numbers are the mean of two to six samples.
Abbreviations: POD, postoperative day; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HPF, high-power field (×400); BrdU, 5-bromo-2′-deoxyuridine; TUNEL, terminal deoxynucleotidyl transferase-mediated biotin-dUTP nick end labeling.

DISCUSSION

Migration of donor MHC class II$^+$ cells after OLTx to recipient lymphoid tissues under tacrolimus induced splenic

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proliferation and subsequent apoptosis of splenocytes without a Th1-type cytokine-rich milieu. This resulted in a Th2-type cytokine-dominant milieu in liver allografts and apoptosis in graft infiltrates. Donor passenger leukocytes expressing MHC class II^+ may play an active role in liver graft acceptance under tacrolimus treatment.

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